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Kit-Wah Lee
University of Nebraska

Robert C. Lommasson
University of Nebraska

The Epidermis of the Blade Joint of *Sorghum Bicolor* (L.) Moench[1]

KIT-WAH LEE and ROBERT C. LOMMASSON[2]

Abstract. The epidermis of several strains of cultivated sorghums were examined microscopically by means of epidermal casts and macerated leaf samples. One variety was examined at several stations in the sheath, blade joint and blade. An account of cell types, their sizes, and their distribution is given for both sheath and blade, and are compared with those of the blade joint. The long-cells of the outer surface of the blade joint are shorter than in other parts of the leaf, silica-bodies are exclusively cross-shaped, and stomata are few in number. An unspecialized area of epidermal cells is located at the very base of the blade in the blade joint region on the upper epidermis. These are transformed meristematic cells. Specialization of cells proceeds from the tip of the leaf and ends in the upper region of the blade joint. The lowest cell specialization may be seen above major vascular bundles. The epidermis of the ligule is similar in general aspect to that of the inner leaf sheath, but no stomata are present. Macro-hairs are present at the base of the abaxial surface of the ligule, but are absent on the adaxial surface.

In general appearance, the leaves of *Sorghum bicolor* are like those of other grasses. They are borne on the culm in two ranks, one at each node. The number of functional mature leaves on a plant when the head is maturing varies with the variety. Each leaf consists of a blade and a sheath. At the junction of sheath and blade there is a prolongation of the sheath called a ligule. From the point where the blade joins the sheath it gradually broadens to the widest part (about one-third the length) and then it tapers toward the apex. The midrib is usually prominent especially on the lower surface. It divides the blade into two asymmetrical halves. At the base of the blade the midrib is very wide, and it tapers gradually toward the apex and disappears altogether before reaching the tip. As the leaf matures the parenchyma tissue of the midrib becomes filled with intercellular spaces thus the midrib has a whitish appearance. The sheath encircles the culm, its margins overlapping alternately in successive nodes. Its outer surface is usually whitish due to the wax deposits. The inner surface of the sheath is often purplish in color due to sap pigments.

The role of leaf anatomy and epidermis have been stressed by Stebbins (1956) in his statement that ". . . the morphological characteristics which reveal more consistently the true interrelationship between species and genera must therefore be sought in microscopic characters of the vegetative organs, such as leaf anatomy and epidermis. . . ."

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[2]Graduate student and professor, respectively, Department of Botany, University of Nebraska, Lincoln, Nebraska.

Artschwager (1930) in studying the epidermis of sugarcane stem observed three patterns of short-cell arrangement among different varieties. Prat (1932) made a study of grass epidermis and compared their classification based on epidermal characters and upon flora characters. Later he (Prat 1936) discussed the systematics of grasses and recognized the distinctions which Avdulov (1931) had pointed out. These included the distinctness of the Bambusoideae from the Festucoideae and the Panicoideae. The epidermal appendages as well as other characters showed the closeness of the chlorodoid and oryzoid grasses to the panicoids rather than to the fectucoid grasses. More recently Prat (1948, 1961) has continued to emphasize the use of epidermal characters in grass systematics. Tateoka, Inoue and Kawano (1959) have made an intensive study of the bicellular microhairs of many grasses.

Materials and Methods

Fresh material was collected from mature plants growing under field conditions at the Agronomy Farm, University of Nebraska or from greenhouse grown plants raised from seed provided by the Seed Laboratory, University of Nebraska. For comparison the fourth leaf below the inflorescence was sampled in all cases. One strain, Standard Broomcorn CI 556, was studied most intensively, and samples were taken from one leaf starting at the sheath (Stations I, II, III) and progressing upward through the blade joint (Station IV) to four samples of the blade (Stations V, VI, VII, VIII) which are 13, 26, 39, and 52 cm. above the blade joint. Station VI was the widest sample taken (ca 5.2 cm. in a leaf 52.2 cm. long). Measurements of cell size and photographs were made from this strain.

In the study of the epidermis the following two techniques were used:

Maceration Technique

Small pieces of leaf tissue were placed in a beaker containing dilute nitric acid to which several crystals of potassium chlorate were added. After several minutes of heating, the epidermis loosely separates from the mesophyll. At this time water is added and the contents are poured into a petri dish partly filled with water. The epidermis is peeled off carefully and dehydrated in 95 percent ethyl alcohol, stained with fast-green, and mounted in Euparol. This is the only method that was successfully used in preparation of material at the blade joint, particularly the ligule.

Casting Technique

A 0.003 inch clear cellulose acetate film about one inch square was placed on a flat surface and a few drops of acetone were spread over its surface. After a few seconds a leaf was pressed onto the film, and allowed to remain two to three minutes until the plastic film had dried

and could be peeled off of the leaf. The film was cut or trimmed as needed and mounted on a slide under a cover glass without the use of mounting medium, but kept in place by adhesive tape. This technique is especially useful in making stomatal counts and has been widely used in recent years.

Observations

Leaf Blade

The epidermis of the leaf of *Sorghum bicolor,* like that of most other grasses, is highly differentiated. The several cell types, together with the stomata, are arranged in longitudinal rows on all leaf surfaces. The lower epidermis of the blade (Figure 2) differs from the upper epidermis (Figure 1) by the absence of bulliform cells.

The characterization of epidermal cells and appendages follows the usage of Metcalfe (1960). Long-cells constitute the greater part of the epidermis. The walls of these cells are sinuous and thick, especially in cells near the leaf blade margin. The outer wall is strongly cutinized. The long-cells which occur near the major vascular bundles are longer and narrower than those in the intercostal areas. Long-cells in the intercostal areas may be from 25 to 70 microns in length and 17 to 24 microns in width. Those near the major vascular bundles (costae) may be 75 to 120 microns long by 10 to 12 microns wide. Directly above the costae the long-cells may be only 15 to 35 microns long by 10 to 14 microns wide. Thus the long-cells directly above a major vascular bundle are significantly shorter than those near the same vascular bundle. The long-cells of the midrib are longer, narrower, and the lateral walls are not as sinuous as those of the lamina. Those on the adaxial surface may be 88 to 155 microns long by 7 to 34 microns wide, while those of the abaxial midrib range from 70 to 130 microns in length by 15 to 20 microns in width.

Short-cells occur in the same files as the long-cells and usually alternate with them. Short-cells usually occur in pairs separated by a single long-cell. A silica-cell and a cork-cell are the usual members of the pair and are always arranged apically and basally, respectively. The silica-cells have but one silica body per cell, and this generalization holds for the whole grass family in contrast to the Cyperaceae where there may be several silica bodies per cell. Silica bodies appear in different characteristic shapes and include the dumbbell, nodular, and cross-shaped types of Metcalfe (1960). The cells are about 15 to 25 microns long by 10 microns wide when they occur above a major vascular bundle, but they are smaller in the intercostal area. Cork-cells are often about 8 microns long by 15 microns wide. In the costal zone pairs of short-cells may lie adjacent to each other by the omission of long-cells. In the intercostal area the silica-cells may be omitted or they may be replaced by a hair. Occasionally prickle-hairs or a mirco-hair may replace a short-cell pair.

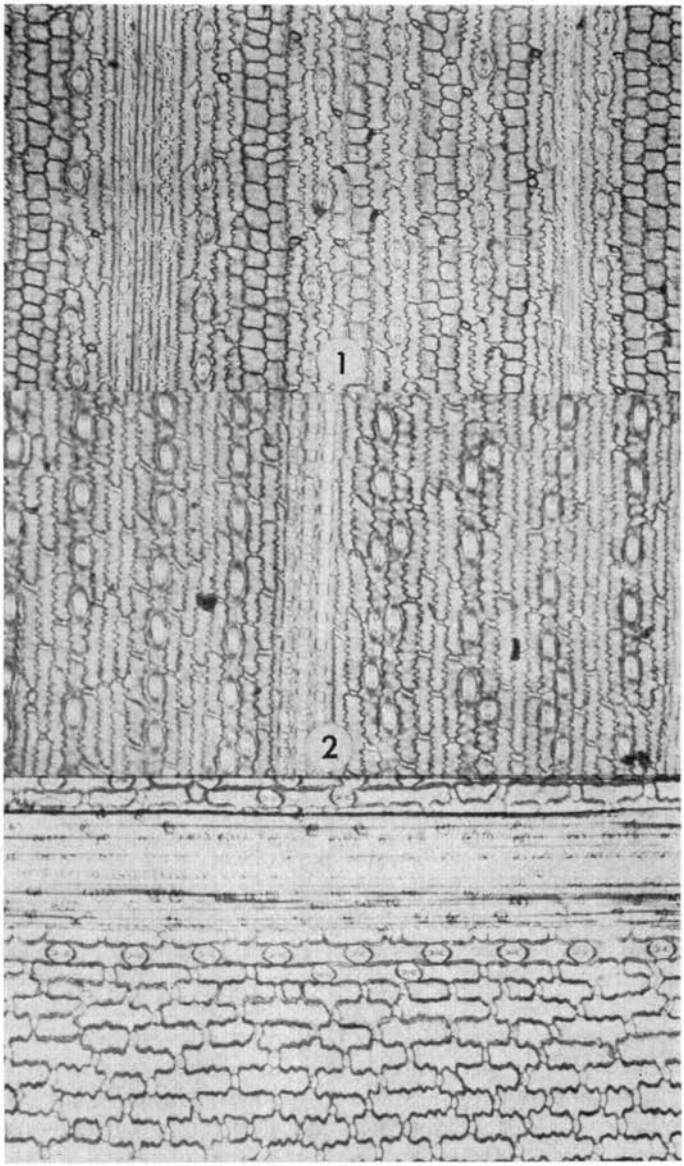

Figure 1. Upper epidermis of a leaf blade of Standard Broomcorn CI 556.
Figure 2. Lower epidermis of a leaf blade of Standard Broomcorn CI 556.
Figure 3. Outer epidermis of the leaf sheath showing costal zone (above) and intercostal zone (below).

Bulliform cells are a specialized type of epidermal cell of the adaxial surface of the blade. These cells occur in strands one to six cells wide between all but the smallest vascular bundles. They are 20 to 60 microns long by 12 to 30 microns wide, their walls are more or less sinuous, and their outer walls are not cutinized, but the cuticle on the surface is longitudinally striated with shallow groves. These cells may penetrate into the leaf 25 to 40 microns.

The stomatal complex in this species, as in all grasses, consists of two guard cells and two subsidiary cells. They occur in one or two files of cells on each side of the larger vascular bundles on both adaxial and abaxial surfaces. Within each file of cells the stomatal apparatus is separated by modified long-cells called interstomatal cells (I.S.C.). Stomatal development follows the pattern described by Flint and Moreland (1946) and by Porterfield (1937). As divisions occur a small distal guard mother cell with large nucleus and dense cytoplasm is cut off from a larger proximal cell which soon becomes vacuolated. The adajacent undifferentiated epidermal cells in the contiguous files divide asymmetrically, each giving rise to a small cell on each side of the guard mother cell. These are the subsidiary cells and are produced after the guard mother cell. A longitudinal division of the guard mother cell produces the two guard cells and schizogenous slit appears between them producing the stomatal aperature. The whole complex of four cells then matures. Stomatal densities vary considerably among the twenty strains of this species which were examined. The adaxial blade counts ranged from 158 to 77 per square milimeter while those of the abaxial surface varied from 233 to 131 per square milimeter. In Standard Broomcorn CI 556 the greatest stomatal density occurs in Station 6 which is also the widest portion of the leaf blade (Table 1).

Table 1

Stomatal Density in the Upper and Lower Epidermises of the Blade of Standard Broomcorn CI 556

Station and Surface	Range per mm.^{2}	Average per mm.^{2}	Standard Deviation	I.S.C. Length in Microns
5 U	60- 86	73	10.0	59
5 L	136-162	153	9.4	55
6 U	94-115	106	8.1	68
6 L	138-173	161	14.8	56
7 U	86-108	100	8.8	67
7 L	138-155	150	8.4	44
8 U	61- 82	74	8.2	78
8 L	124-145	136	8.4	71

The epidermal appendages consist of macro-hairs, micro-hairs, and prickle-hairs. Macro-hairs are large unicellular hairs with swollen bases and thick walls. They are found commonly in the ligular region of the upper epidermis of the leaf blade. Micro-hairs are bicellular.

The basal cell is shorter, yet its wall is thicker, than that of the distal cell which Metcalfe (1960) has suggested is secretory. These cells are usually found in the intercostal zones of both adaxial and abaxial surfaces of the blade. They arise between long-cells, or as a member of a short-cell pair and take the place of a silica-cell. Sometimes they may be found between bulliform cells. These hairs occasionally may be found on the abaxial surface of the sheath. Prickle-hairs are unicellular, short, and sharp pointed. Their bases are much swollen and thick walled. They have been described as being of several types which vary in the size and shape of the base. Their distribution is often restricted to certain areas, e.g., the largest often occurring in the leaf margin, another type found principally along the midrib.

Leaf Sheath

The outer surface of the leaf sheath is composed of short-cells, long-cells, and stomata arranged in longitudinal files (Figure 3). The long-cells range from 15 to 25 microns in length and their walls are sinuous. They normally alternate with short cells, but occasionally a micro-hair intervenes between adjacent long-cells. The stomata form in only one or two files of cells adjacent to the costal region and are separated by one or two interstomatal cells. The stomatal density is greatly decreased from that of the blade in which files of cells on each side of most of the longitudinal vascular bundles contained stomata.

The epidermis on the inner surface of the leaf sheath (Figure 4) is completely different from that of the outer surface. It consists of elongate cells with nearly straight, thin, lateral walls. The elongate cells of the intercostal zone are shorter and wider than those of the costal zone. The intercostal cells range from 18 to 40 microns in length and are 10 microns wide, whereas those in the costal zone are about 40 to 60 microns in length and are 7 microns wide. Stomata are also present in one or two files bordering the costal region. There are two or more interstomatal cells separating stomata. The density of stomata of the inner epidermis of the leaf sheath is only about 20 per square milimeter. Short square or rectangular cells found in files which contain stomata may be undifferentiated guard mother cells. The inner epidermis lacks typical long-cells, short-cells, or prickle-hairs.

Blade Joint

The area of juncture of blade and sheath is called the blade joint. The gross structure of this area which includes the ligule and the dewlap has been discussed for *Sorghum* by Artschwager (1948). The abaxial side of this region has an epidermal pattern similar to that of the abaxial epidermis of the blade and sheath except that neither costal nor intercostal areas are differentiated (Figure 5). In this region long-cells, short-cells, and stomata are present. The long-cells have thick walls which are not as sinuous as those in the blade. Their length

is considerably less than that of other long-cells, ranging from 15 to 50 microns while their width is about normal, 12 to 25 microns. The short-cells are usually distributed in pairs separated by a single long-cell. Occasionally the silica-cell of a pair may be absent. In this region all of the silica-bodies within the silica-cells are cross-shaped. The stomata are few in number and are so distantly spaced that they appear to be randomly dispersed rather than being in specific files. Hairs, though abundant in the region of the blade joint, are absent from an area on the abaxial side which is comparable to the base of the ligule on the adaxial side. Above and below hairs become numerous a short distance away. They become visibly thick on the base of the blade but usually not so on the top of the sheath.

On the adaxial side of the blade joint there is a ligule which in cross section was found to be composed of epidermis and parenchyma cells. The epidermises of both sides of the ligule are composed entirely of elongate cells (Figure 6). Neither short-cells nor stomata are present. The lateral walls of the ligule epidermal cells are not sinuous, and the cell contents consist of numerous granules. This latter condition may account for the non-green coloration of the ligule and dewlap. The cells on the adaxial surface of the ligule are larger (15 to 20 microns by 60 to 100 microns) than those of the abaxial surface (7 to 10 microns by 50 to 80 microns). In addition to these long-cells, there are many macro-hairs present at the base of the outer surface of the ligule, extending parallel along its surface and partially obscuring the abaxial epidermal cells.

At the extreme basal region of the blade where it joins the ligule the upper epidermis of the blade joint is composed of cells which are morphologically unique to the epidermal system of the shoot. In this region the cells are square to irregular in shape, often being wider than they are long. These are small cells and the most unspecialized of any mature epidermal cells to be found on the leaf. They are essentially arranged in files and are distinctive because of their thick walls. Neither stomata nor other specialized epidermal cell types are found in this area. The unspecialized area may extend distally about 1.5 mm. from the base of the ligule. The farther away from this basal area toward the leaf tip, the more abundant are the hairs. At the distal part of this region, cell specialization occurs first in the area which overlies a major vascular bundle (Figure 8). The long-cells and short-cells are clearly defined while the adjacent cells remain irregular in shape and small in size.

Discussion

The intercalary meristem at the base of the leaf primordium becomes divided into two regions of elongating cells by the early differentiation of mature cells of the sheath. The final development of the epidermis

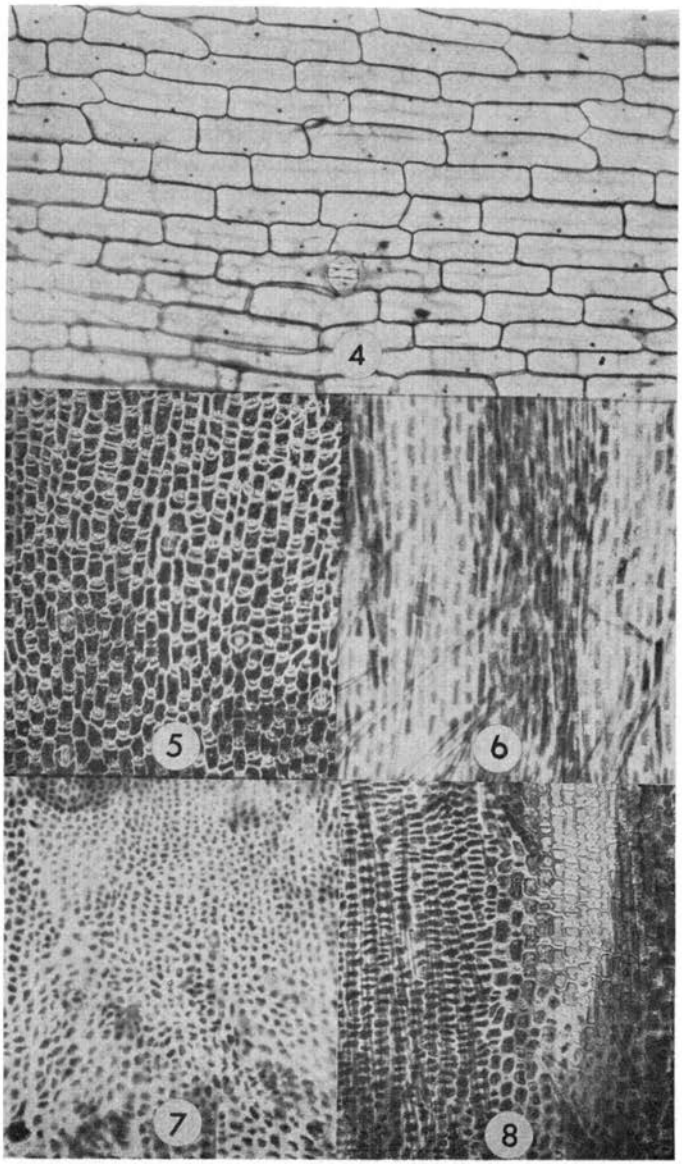

Figure 4. Inner epidermis of the upper region of the leaf sheath showing unspecialized elongate cells and one stomate.

Figure 5. Abaxial epidermis of a blade joint showing the lack of elongation in this area as indicated by the shortness of the long-cells.

Figure 6. Adaxial epidermis of a ligule. The trichomes shown here are not attached to this surface.

Figure 7. Undifferentiated cells of the adaxial epidermis of the base of the blade. This area is a basal part of the blade joint.

Figure 8. Distal part of the unspecialized area of the adaxial epidermis of the base of the blade. The beginning of normal epidermal differentiation in a costal region is shown in the upper right hand area.

of the elongating area at the base of the blade is the main concern of this paper.

Booth (1964) suggested ". . . with the addition of growth hormones, the long-cells increase rapidly while short-cells seem to remain relatively unchanged. . . ." Stebbins (1966) has suggested that "mitosis inducing influences" arising from the reproductive axis and traveling through the abaxial epidermis of the sheath are unable to cross the blade joint region into the blade. He suggests that the procambium of the vascular bundles produces a "mitosis-inducing substance," and that the elongation promoting substance arises from the leaf tip. We have pointed out that long-cells of the leaf blade which lie close to the costal region are longer than those in the intercostal region. This may be due to the influence of the cell-elongating materials translocated downward from the leaf tip. Those cells just above the vascular bundles and the interstomatal cells are shorter than other long-cells. It may be that these cells are influenced to continue divisions longer by the "mitosis-inducing substance" from the procambium of the vascular bundles and the special meristematic activity in files of stomata producing cells.

Maturation influences must be associated closely with the vascular bundles since differentiation extended down from the leaf tip into the blade joint region farther along major vascular bundles. The unspecialized nature of the adaxial surface of the base of the blade might be thought of as simply a remnant of an elongating region whose walls mature before complete elongation possibilities have been exhausted.

Cytoplasmic gradients have been suggested (Stebbins and Shah, 1960) as allowing stomatal development by lateral divisions of adjacent cells in the production of subsidiary cells of the stomatal complex. The over-all longitudinal polarities within the leaf must influence the distal and proximal differentiation of silica-cells and cork-cells, respectively.

The development of the ligule has been studied by Philipson (1935) and Sharman (1941). They agreed that the ligule arose by rapid periclinal divisions in a few young epidermal cells at the union of the future blade and sheath. The nature of the ligule has not been examined here but Philipson (1935) concluded that ". . . the ligule may be said to consist of the free upper border of the sheath and a median upgrowth of the adaxial epidermis of the leaf. . . ." In this study it has been indicated that the epidermis of the ligule, both adaxial and abaxial, is similar to the adaxal surface of the sheath more than to epidermal structures of the blade.

The increased number of stomata and the shorter interstomatal cells although somewhat related to each other are undoubtedly also related to the environmental influences. The widest part of the blade has the greatest number of stomatal pores indicating an increasing number of

files of stomatal producing cells in response to the forces causing general increase in width of the leaf.

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